On peroxidase activity of Mammalian Glutathione Peroxidase

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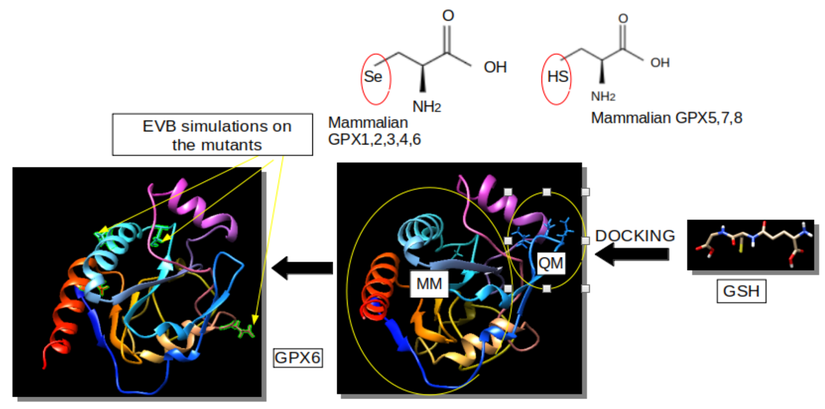
Abstract

The biological effects of selenium are largely mediated by selenium-containing proteins (selenoproteins) [4]. Different isoforms of Glutathione Peroxidase (GPX) have been labeled as potential targets to control or cause the oxidative stress during cancer evolution. [5] [6] In particular, eight different cysteine and selenocysteine containing isoforms of Glutathione Peroxidase (GPX1-8) isoforms have been identified in humans.[7]. The relationships between the mechanism and the structure is not entirely understood, although it has been shown that catalytic activity of several reconstructed ancestral structures of GPX6 recover their oxidative activity when the active site is mutated from Cys to Sec. All this results have led us to propose using a combination of QM/MM, empirical valence bond (EVB) calculations and structural bioinformatics tools to unravel sequence-structure-function relationships in GPX6 and its orthologs.



QUESTION

There appears to exist an evolutionary shift from Sec-containing into Cys-containing sequences in the glutathione peroxidase (GPX) proteins family. A relevant example is GPX6, that contain one of the two residues depending on the mammal species. Due to this shift, the peroxidase activity in lost. Has loosing of peroxidase activity due only to the substitution of Se by S in the active site or do have the accompanying mutations an active role in this process?

HYPOTHESIS

We hypothesize that the lost may be due to the accumulation of mutations and not to a single central one. We will focus on understanding both phylogeny and enzymatic mechanism in GPX6 (Sec- or Cys-containing).

OBJECTIVE

DONE: To explore the mode of interaction between GPX6 and the glutathione cofactor in ancestral to modern GPX6.

IN PROGRESS: To understand the enzymatic mechanism of GPX6 and its mutations.

FUTURE: To create a model that integrates bioinformatics, computational biochemistry tools and a machine learning models to propose the most likely evolutionary pathway by GPX6.

Introduction

1

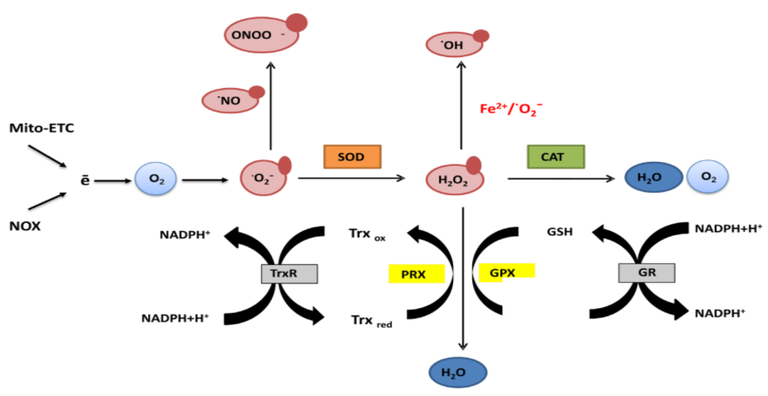
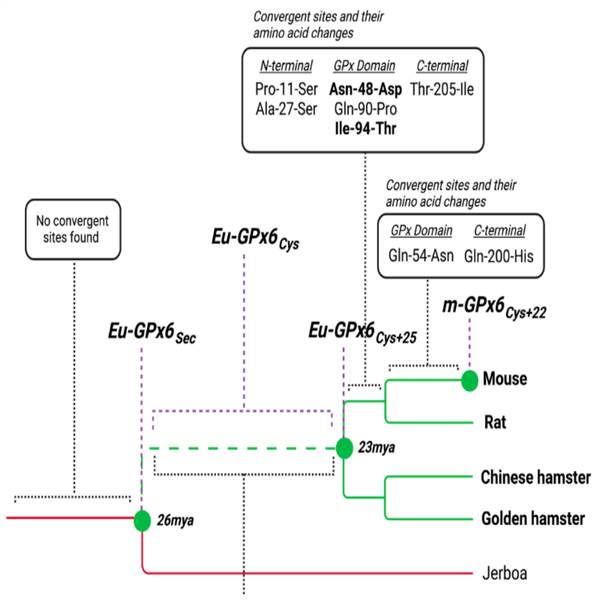
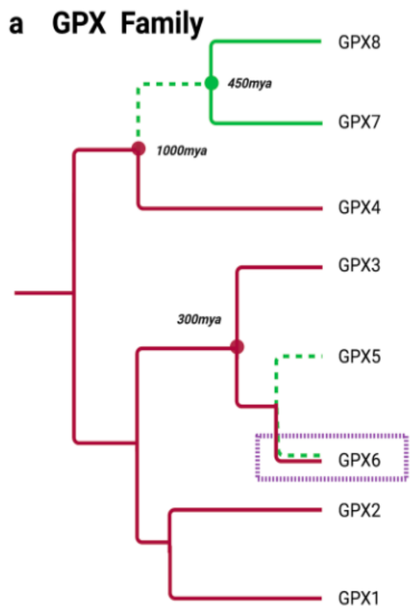


Fig 1. Generation and scavenging of reactive oxygen species (ROS). [1]

2a



2b



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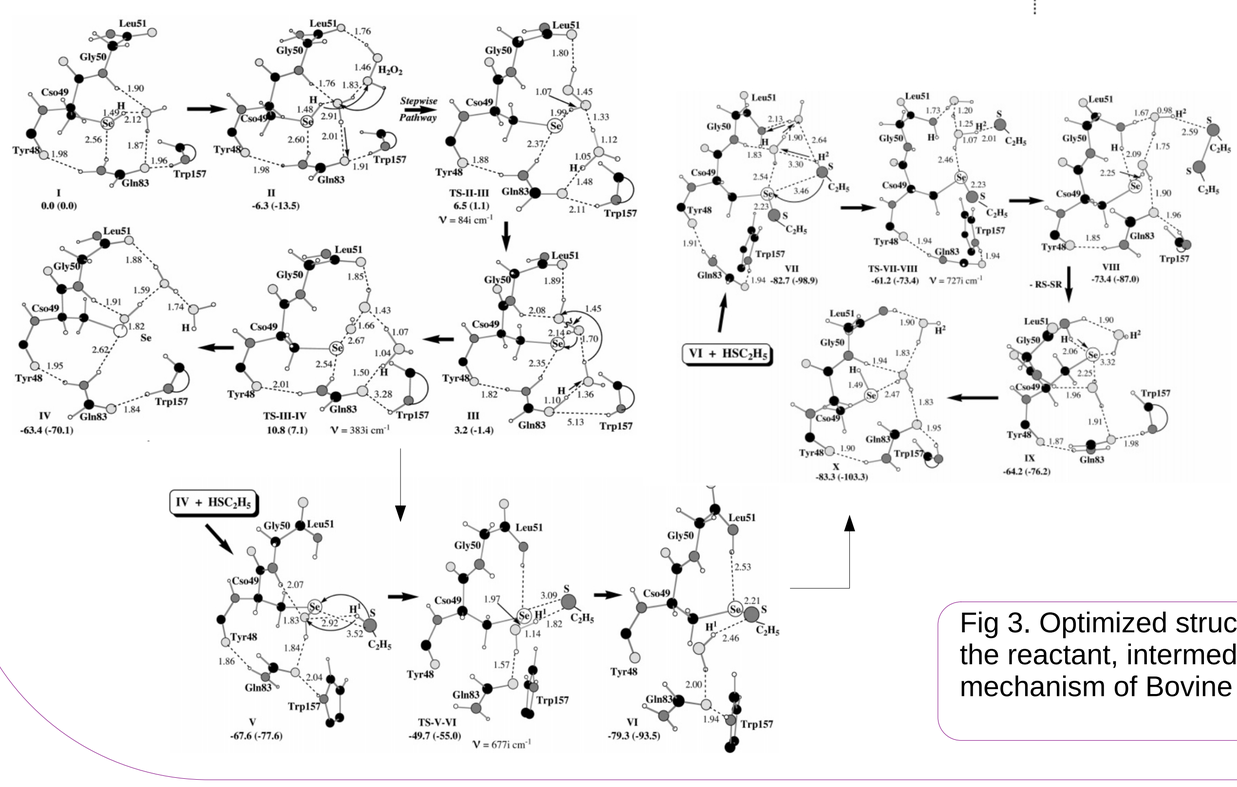
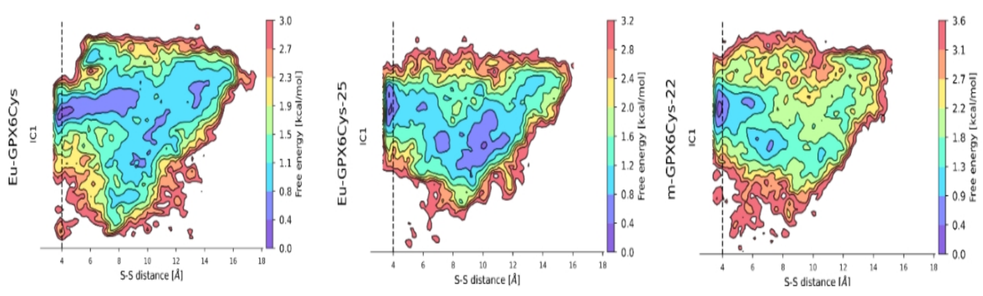


Fig 3. Optimized structures and energies in kcal/mol] of the reactant, intermediates and, TS for the stepwise mechanism of Bovine GPX.[2]

Fig 2a : Topology of the phylogeny of the Eumuroida GPX6Cys clade, green branches,with the Jerboa GPX6Sec lineage red branch and the basal Eumuroida lineage, dashed green branch.[3]
Fig 2b : The phylogeny of the GPX family in Eukaryotes (based on Mariotti et al., 2012). In red, GPX6 Sec branches. In green, GPX6Cys ones. Dashed green branches represent GPX6 Cys lineages where Sec was lost. [3]

Results and Conclusion

4a



1. These are the results of the authors of the poster where the computational analysis suggests that the binding of GSH and overall structures of the enzymes have not been adversely affected by the involvement of Cys.[3]
2.The conservation of GPX6-glutathione interactions, despite the loss of peroxidase activity may suggest additional functional roles of GPX6 still to be described. [3]

4b

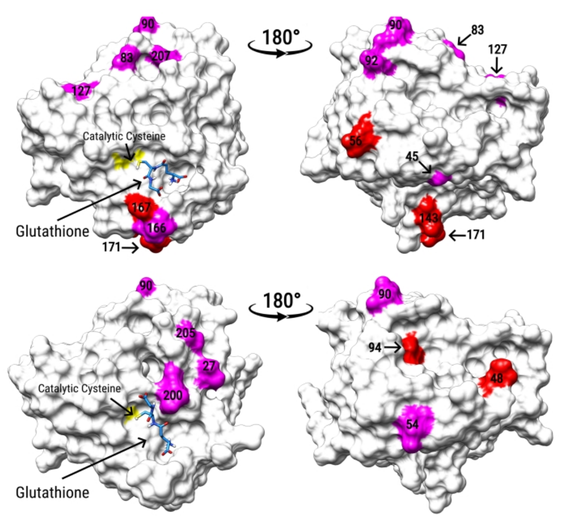


Figure 4a - Free energy profiles for the docking of the glutathione dimer to ancestral and modern GPX6 Cys proteins. Figure 4b - Convergence patterns (Fig 2a) from Eu-GPX6Cys to Eu-GPX6Cys+25 (top) and from Eu-GPX6Cys+25 to m-GPX6Cys+22 (Mouse-GPX6) (bottom). The catalytic cysteine (yellow) is shown with the glutathione best binding energy conformation. [3]

Further Work

Convergent sites and their amino acid changes

N-terminal	GPx Domain	C-terminal
Met-27-Ala	Leu-45-Asn	Lys-166-Glu
	Lys-56-Gln*	**Gln-167-His**
	Val-63-Ile	**Glu-171-Asp**
	Thr-70-Ser	Lys-207-Gln
	Sec-72-Cys	
	Ala-83-Thr*	
	His-90-Gln	
	Asp-92-Asn	
	Phe-127-Tyr	
	Lys-143-Asn	

QM (GAMESS) and QM/MM (pDynamo) methods to unravel the reaction mechanism in modern Cys- and Sec containing GPX6. EVB simulations (Q6, AMBER ff) to understand the effect of mutations in GPX6, comparing current orthologs and branches in the evolutionary pathway. The above preliminary results show a well defined path of mutations. So, our next goal is now to run EVB simulations with high convergence confidence on the different mutants. For which we will use the Q6 program to run the EVB calculations for each of the specific mutants. Initial relaxation simulations with OpenMM and AMBER ff will be run on the ancient reconstructed Sec-GPX6. Structures for the GPX6 orthologs in the above results were built using AlphaFold2.

Methods

Current status

1.Alphafold2 for protein structure reconstruction from ancestral sequences.
2.Molecular dynamics simulations (OpenMM, AMBER ff) to explore the conformational landscapes of GPX6 and its complexes with glutathione disulfide.
3.Protein-ligand binding energy landscape explorations was done using the PELE software (Protein Energy Landscape Exploration)
4.Time-structure Independent Component Analysis (TICA) was performed with the PyEMMA library.

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